# Interferon-γ Coordinately Upregulates Matrix Metalloprotease (MMP)-1 and MMP-3, But Not Tissue Inhibitor of Metalloproteases (TIMP), Expression in Cultured Keratinocytes

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**Matrix metalloproteases (MMP) constitute a family of proteolytic enzymes degrading extracellular matrix components. Their activity is inhibited by tissue inhibitors of metalloproteases (TIMP). Previous studies have demonstrated that various cytokines can modulate MMP and TIMP gene expression. In this study, we demonstrate that interferon-γ coordinately upregulates MMP-1 (interstitial collagenase) and MMP-3 (stromelysin-1) gene expression in cultured keratinocytes, as determined at the mRNA steady-state levels, and this effect is dependent on on-going protein synthesis. In contrast, there was no effect on TIMP-1 gene expression. Enhanced MMP-1 expression by IFN-γ was also demonstrated at the protein level by Western analysis. Transient transfections with MMP-1 and MMP-3 promoter/reporter gene constructs revealed no response to IFN-γ, whereas incubation of keratinocytes with this cytokine appeared to stabilize the MMP-1 mRNA, resulting in reduced turnover of the transcript. These data suggest that IFN-γ enhances MMP gene expression at the post-transcriptional level. The altered MMP expression by IFN-γ without concomitant effect on TIMP gene expression potentially leads to imbalance between these proteases and their inhibitors, and enhanced proteolytic activity may play a role in the remodeling of cutaneous tissue involving inflammatory processes, such as wound healing.** ***Key words: matrix metalloproteases/tissue repair/extracellular matrix turnover/inflammatory mediators. J Invest Dermatol 104: 384–390, 1995***

Matrix metalloproteases (MMPs) constitute a family of structurally related proteolytic enzymes, yet their specific activities are divergent [1,2]. For example, MMP-1 (interstitial collagenase) is the major enzyme responsible for degradation of extracellular fibers comprised of collagen types I, II, or III [1]. MMP-3 (stromelysin-1) depicts a broad spectrum of proteolytic activities, including degradation of various gelatins, proteoglycan link protein, fibronectin, and laminin, as well as native collagens type III, IV, V, and IX [1]. Furthermore, MMP-3 is required for maximal activation of MMP-1 in physiologic situations [3].

The activities of MMP-1 and MMP-3 are regulated by a family of tissue inhibitor of metalloproteases (TIMP) [1–5]. The major inhibitor of MMP-1 and MMP-3 is TIMP-1, a 28.5-kDa glycoprotein, which forms 1:1 stoichiometric complexes with these proteases [1,6]. Thus, the relative levels of TIMP-1 regulate the activity of MMPs in tissue. Any imbalance between the proteolytic activities and their inhibitors could potentially lead to a pathologic condition, as exemplified by degradation of articular connective tissue in arthritic diseases or accumulation of collagen in cutaneous collagenomas due to deficient collagenase activity [7,8].

Previously, a variety of cytokines and growth factors have been shown to regulate the expression of MMP-1 and MMP-3, as well as TIMP [2,9]. This modulation is often selective, and different cytokines can alter the balance between the active proteases and their inhibitors. One of the cytokines previously shown to modulate the activity of MMP-1 and MMP-3 is interferon-γ (IFN-γ), a physiologic mediator synthesized and released primarily by helper T-lymphocytes [10]. Previous studies have demonstrated that IFN-γ decreases the activity of MMP-1 in rheumatoid synovial fibroblasts, human articular chondrocytes, and human alveolar macrophages in culture [11–13]. Besides inhibiting constitutive expression of MMP-1, IFN-γ also counteracts interleukin-1–induced MMP-1 production [12]. Similarly, the expression of MMP-3 in fibroblast cultures was found to be inhibited by the addition of IFN-γ, whereas the levels of TIMP were essentially unaltered [4]. IFN-γ also potently suppressed lipopolysaccharide-induced production of MMP-3 in human macrophages, whereas inhibition of TIMP synthesis required fifty- to a hundredfold higher concentrations of this cytokine [13]. Thus, these previous studies suggest that IFN-γ is primarily inhibitory for MMP activity in *in vitro* culture systems utilizing a variety of different cell types.

Human epidermal keratinocytes in culture have recently been shown to synthesize various MMPs, including MMP-1, MMP-2 (72-kDa gelatinase), MMP-3, and MMP-9 (92-kDa gelatinase)

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[14–16]. In addition, cultured keratinocytes constitutively express TIMP [15]. MMP-1 synthesis was shown to be enhanced by keratinocytes cultured on type I or type IV collagen [17], whereas the constitutive synthesis of MMP-9 was enhanced by type I collagen and decreased by type IV collagen matrices [18]. Thus, the extracellular microenvironment, as well as soluble mediators, such as IFN-γ, can alter the activities of tissue MMPs.

In this study, we have examined the expression of MMP-1 and MMP-3, as well as of TIMP, in human epidermal keratinocytes in culture.

## MATERIALS AND METHODS

**Northern Analyses** For RNA isolation, human adult keratinocytes were cultured in serum-free, low-calcium (0.15 mM) medium, supplemented with epidermal growth factor, hydrocortisone, insulin, and bovine pituitary extract (keratinocyte growth medium, Clonetics Corporation, La Jolla, CA). Some cultures were incubated with recombinant human IFN-γ (Boehringer Mannheim, Indianapolis, IN), in concentrations indicated in *Results*. Cycloheximide, 10 μg/ml, was added to some cultures to inhibit on-going protein synthesis. For Northern analysis, RNA was isolated from cultured cells by a single-step extraction procedure [19]. Total RNA, 30 μg per lane, was fractionated on 1% agarose gel and transferred to nitrocellulose filters, and Northern hybridizations were performed with the cDNAs labeled radioactive with $\alpha[^{32}P]$GTP and $\alpha[^{32}P]$CTP by nick translation [20]. The following cDNAs were used for hybridizations: for MMP-1 (interstitial collagenase), a 2.0-kb human cDNA [21]; for MMP-3 (stromelysin-1), a human 1.5-kb cDNA [22]; and for TIMP, a 0.7-kb cDNA, kindly provided by Dr. Carmichael, Synergen Corp., Boulder, CO. As a constitutively expressed control, a rat glyceraldehyde-3-phosphate dehydrogenase cDNA [23] was used for hybridization. The hybridization and washing conditions were the same as reported previously [24].

**Western Analysis** To determine the amount of immunoreactive collagenase, the cell culture media after 24 h of incubation with or without IFN-γ were concentrated by ultrafiltration (Centricon, Amicon, Beverly, MA). Samples corresponding to the same volume of the original medium were electrophoresed on 10% sodium dodecylsulfate–polyacrylamide gels, and the proteins were transferred to nitrocellulose filters by electroblotting. The immunoreactive collagenase in the filters was detected by incubation with a polyclonal rabbit anti-human collagenase antibody [25] (kindly provided by Dr. William Parks and Dr. Howard Welgus, Jewish Hospital, Washington University Medical Center, St. Louis, MO). The secondary antibody consisted of goat anti-rabbit IgG conjugated with alkaline phosphatase, and the antibody complexes were visualized by a commercial kit (Promega, Madison, WI).

**Transient Cell Transfections** Promoter-CAT constructs for MMP-1 and MMP-3 were used for transient transfections of cultured keratinocytes utilizing a liposome-based commercial kit (DOTAP, Boehringer Mannheim). The MMP-1 construct, pCLCAT3 consisted of 3.8 kb of collagenase promoter region linked to the chloramphenicol acetyl transferase (CAT) reporter gene [26]. The MMP-3 construct, 4+CAT, consisted of 1.3 kb of the stromelysin-1 5′-flanking DNA, also linked to the CAT reporter gene [22]. The promoter-CAT constructs were co-transfected with 2 μg of a RSV/β-galactosidase construct (Promega). IFN-γ was added to some cultures 1 h after transfection. Parallel cultures were transfected with a thymidine kinase (TK)–CAT construct, or a construct that contained three AP-1 consensus sequences (5′-TGAGTCA-3′) cloned in tandem in front of the TK promoter [27], a construct referred here as $[TRE]_3$TK-CAT (kindly provided by Dr. Steven Frisch, Washington University, St. Louis, MO). After 24 h of incubation, CAT activity was determined as described previously [28]. β-galactosidase activity was determined in the same cell extracts [20], and CAT activities were corrected for β-galactosidase activity to normalize for small differences in the transfection efficiency.

**Gel Mobility Shift Assays** Nuclear proteins were isolated from keratinocytes incubated with or without IFN-γ (100 U/ml) for varying time periods up to 24 h [29]. For the binding assay, a 20-bp double-stranded oligomer that contained the collagenase AP-1 consensus sequence (see above), labeled radioactive by $\alpha[^{32}P]$ATP, was used as a probe for protein binding. The oligomer, containing approximately $5 \times 10^4$ cpm of radioactivity, was incubated with 8 μg of the nuclear protein extract, and the DNA-protein complexes were fractionated on 4% polyacrylamide gel containing 0.4 × TBE under non-denaturing conditions, as described elsewhere [30]. The radioactive oligomer-protein complexes were visualized by autoradiography by exposure of the gels to X-ray films with intensifying screens at −70°C.

**RNA Stability Assay** To determine the stability of MMP-1 mRNA, parallel keratinocyte cultures were incubated without or with IFN-γ (100 U/ml) for 24 h, as indicated above. At this point (time 0) the cells were rinsed with phosphate-buffered saline, and fresh medium devoid of IFN-γ but containing 60 mM DRB, an inducer of premature termination of transcription [31], was added. Total RNA was isolated from parallel cultures at different time points up to 48 h of incubation. The MMP-1 mRNA levels were then determined by Northern hybridizations, followed by scanning densitometry, as described above.

## RESULTS

**IFN-γ Enhances MMP-1 and MMP-3 mRNA Levels** Expression of the MMP-1, MMP-3, and TIMP genes was first examined in cultured keratinocytes by assay of the corresponding mRNA steady-state levels by Northern analyses. In cultures incubated in serum-free medium containing 0.15 mM calcium, low, yet clearly detectable, levels of mRNAs for all three genes were detected **(Fig 1)**. Incubation of the cultured cells with IFN-γ (100 U/ml) resulted in a significant, time-dependent increase in MMP-1 and MMP-3 mRNA levels **(Fig 1)**. The maximum enhancement was noted at 12 h of incubation in cases of both MMP-1 and MMP-3 mRNAs **(Fig 1*B*)**. In contrast, the mRNA levels of TIMP were unaltered by IFN-γ **(Fig 1*B*)**. Subsequent incubation of keratinocytes with varying concentrations (up to 1000 U/ml) of IFN-γ for 24 h indicated a marked enhancement, up to approximately eightfold, of collagenase mRNA levels **(Fig 2*A*)**, and maximum stimulation was reached already with 10 U/ml of IFN-γ **(Fig 2*B*)**. Similar increases were noted in stromelysin mRNA levels, whereas the mRNA levels for TIMP were unaltered in these concentrations **(Fig 2*B*)**. Thus, there is a coordinate upregulation of MMP-1 and MMP-3 in cultured keratinocytes by IFN-γ, and this enhancement is selective in that the TIMP mRNA levels are unaltered.

To examine whether the enhancement of MMP-1 and MMP-3 gene expression, as detected at the mRNA level, is dependent of on-going protein synthesis, cell cultures were pre-incubated with cycloheximide (10 μg/ml) 1 h prior to addition of IFN-γ. As indicated above, addition of IFN-γ alone (100 U/ml) to keratinocyte cultures significantly increased the MMP-1 and MMP-3 mRNA levels in the absence of cycloheximide **(Fig 3)**. Addition of cycloheximide to parallel cultures resulted in a marked reduction in the constitutive expression of MMP-1 and MMP-3, respectively **(Fig 3*B*)**. Furthermore, the addition of cycloheximide prevented the enhancement of the corresponding mRNA levels by IFN-γ **(Fig 3)**. It should be noted that the glyceraldehyde-3-phosphate dehydrogenase levels in all cultures were essentially unaltered **(Fig 3*A*)**. Thus, the upregulation of MMP-1 and MMP-3 gene expression, as determined at the mRNA level, is dependent on active protein synthesis.

**Enhanced MMP-1 Expression by IFN-γ at the Protein Level** To demonstrate that the enhancement of MMP-1 mRNA levels by IFN-γ is accompanied by enhanced synthesis of the corresponding protein, Western analysis of the collagenase protein in medium of keratinocytes cultured in the presence of 0–100 U/ml of IFN-γ was performed. Western blot analyses revealed in control cultures a characteristic doublet consisting of collagenase precursor polypeptides of approximately 62 and 57 kDa in size **(Fig 4)**. The intensity of these two bands was significantly enhanced in the presence of 10 or 100 U/ml of IFN-γ **(Fig 4)**. Thus, the elevated mRNA levels for MMP-1 correlate with enhanced synthesis and secretion of immunodetectable interstitial collagenase in keratinocyte cultures.

**Evidence for Post-Transcriptional Regulation** To examine the possibility that the elevated mRNA levels for MMP-1 and MMP-3 result from enhanced transcriptional activity of the corresponding genes, promoter-reporter gene (CAT) constructs were used in transient transfections of cultured keratinocytes. One hour following the transfection with a MMP-1/CAT construct, IFN-γ, in concentrations varying from 0 to 1000 U/ml was added to parallel duplicate cultures. Assay of CAT, as an indicator of the promoter activity, did not reveal any differences in the presence of

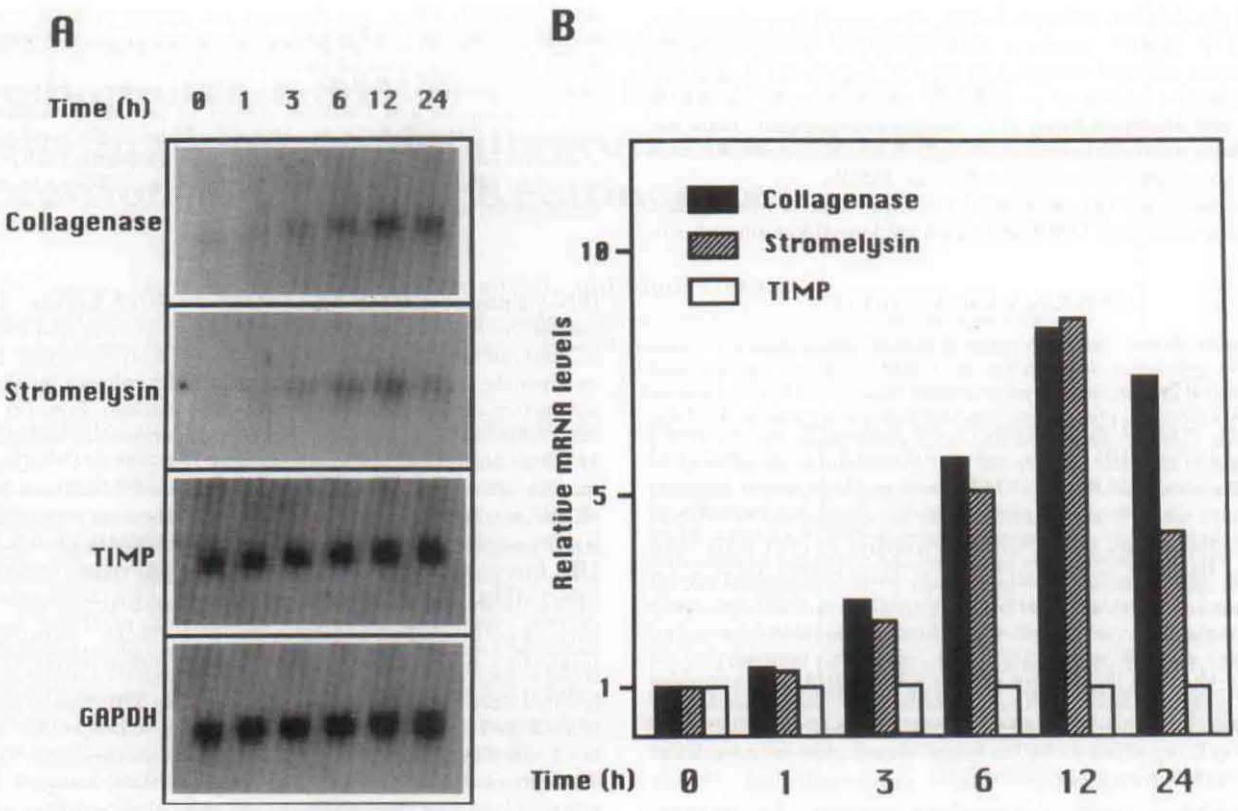


**Figure 1. Time-dependent enhancement of collagenase (MMP-1) and stromelysin (MMP-3) gene expression by interferon-γ (IFN-γ).** Epidermal keratinocytes in culture were incubated with IFN-γ for the time periods indicated. Total RNA (30 μg) was fractionated on 1% agarose gel, transferred to nitrocellulose filters, and hybridized successively with MMP-1, MMP-3, TIMP, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNAs labeled radioactive by nick translation (*A*). Scanning densitometry of the autoradiogram demonstrates a marked enhancement of MMP-1 and MMP-3 mRNA steady-state levels, with the maximum enhancement noted at 12 h of incubation after correction for GAPDH levels in the same RNA preparations (*B*). In contrast, IFN-γ had no effect on the TIMP mRNA levels.

varying concentrations of IFN-γ **(Fig 5)**. Similar results were obtained when MMP-3 promoter/CAT construct was used for transient transfections (not shown). Thus, these results suggest that the elevated mRNA levels for MMP-1 and MMP-3 elicited by IFN-γ may result from regulation at the post-transcriptional level.

In transient transfections of cultured keratinocytes, it was noted that the constitutive activity of MMP-1 promoter/CAT construct was relatively high in control cultures. Because previous studies have suggested that the AP-1 binding sequence (TRE) may serve as an enhancer of collagenase or stromelysin promoter activity [1,2], the ability of AP-1 to upregulate a heterologous promoter, thymidine kinase (TK), was examined in keratinocyte cultures. As shown in **Fig 6*A***, TK-CAT alone gave a relative weak signal, whereas transfections with the $[TRE]_3$TK-CAT construct resulted in a markedly enhanced CAT activity **(Fig 6*A*)**. However, addition of IFN-γ to parallel cultures transfected with the $[TRE]_3$TK-CAT construct did not further enhance the CAT activity **(Fig 6*B*)**. Furthermore, gel mobility shift assays indicated that control keratinocyte cultures contained significant TRE binding activity (AP-1), but incubation of these cells with IFN-γ (100 U/ml), up to 24 h, did not appreciably alter the level of this activity, as detected by radiolabeled oligomer/protein complex in gel mobility shift assay **(Fig 6*C*)**. These data further emphasize the possibility of post-transcriptional level of regulation of MMP-1 and MMP-3 expression by IFN-γ in cultured keratinocytes.

**Stabilization of MMP-1 mRNA by IFN-γ** To examine the effect of IFN-γ on MMP-1 mRNA stability and turnover, keratinocyte cultures were first incubated with (100 U/ml) or without IFN-γ for 24 h. DRB, an inhibitor of transcription, was then added, and the MMP-1 and glyceraldehyde-3-phosphate dehydrogenase mRNA steady-state levels were determined by Northern hybridizations at different time points. In cultures incubated without IFN-γ the MMP-1 mRNA steady-state levels, after correction for glyceraldehyde-3-phosphate dehydrogenase transcript levels, declined in a time-dependent manner, and an approximately 50% reduction from the control levels (time 0 h) was noted between 8 and 12 h of incubation **(Fig 7)**. In cultures incubated first with IFN-γ, there was no reduction of MMP-1 mRNA levels during the first 8 h following addition of DRB **(Fig 7)**. In subsequent time points, the MMP-1 mRNA levels were reduced as compared to the 0-h cultures incubated with IFN-γ, but the levels remained approximately twofold higher at 24 h, as compared to cultures incubated without IFN-γ **(Fig 7)**. These data provide evidence for initial stabilization of MMP-1 with IFN-γ.

## DISCUSSION

The results of this study demonstrate uncoordinate regulation of two MMPs critical for degradation of the extracellular matrix of connective tissue, and their inhibitor, TIMP, by IFN-γ in human epidermal keratinocytes in culture. Specifically, the MMP-1 and MMP-3 mRNA levels were markedly, up to eightfold, increased by IFN-γ in a time- and dose-dependent manner. In contrast, IFN-γ in concentrations tested (0–1000 U/ml) did not alter the TIMP mRNA levels. The enhancement of MMP-1 gene expression by IFN-γ was also demonstrated at the protein level by Western immunoblot analysis of the secreted proteins.

Previous studies have indicated that the expression of MMP-1 and MMP-3 is significantly modulated in cultured fibroblasts [2]. However, in a distinct contrast to the findings demonstrated in our study in keratinocyte cultures, IFN-γ significantly decreased the

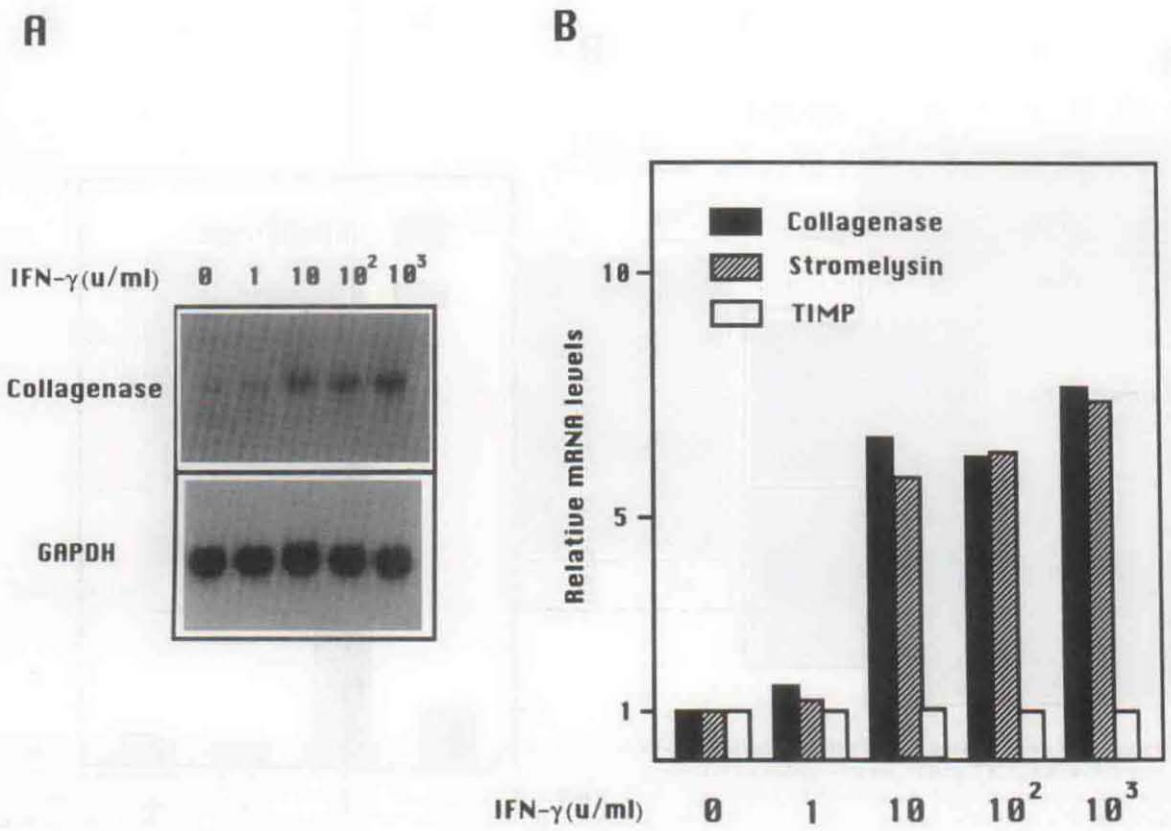


**Figure 2. Dose-dependent enhancement of collagenase (MMP-1) mRNA levels by IFN-γ.** Cultured keratinocytes were incubated with IFN-γ in concentrations indicated for 24 h. Collagenase mRNA and glyceraldehyde-3-phosphate dehydrogenase steady-state levels were determined by Northern hybridizations (*A*). In addition, stromelysin (MMP-3) and TIMP mRNA steady-state levels were determined by re-hybridization of the same filters, as in **Fig 1** (not shown). Scanning densitometry indicated that significant increases in the collagenase and stromelysin mRNA levels were noted already with 10 U/ml of IFN-γ, and further enhancement was noted in the presence of 1000 U/ml (*B*).

expression of the corresponding genes in fibroblast cultures. Similar down-regulation of collagenase or stromelysin expression by IFN-γ has also been previously reported in human articular chondrocytes and alveolar macrophages [11–13]. Thus, the upregulation of collagenase and stromelysin gene expression by IFN-γ, as determined at the mRNA steady-state levels, and in case of collagenase at the protein level as well, appears to be unique to human keratinocytes among the cell types tested thus far.

The level of regulation of MMP-1 and MMP-3 gene expression by IFN-γ appeared to be post-transcriptional. In support of this conclusion were the observations that keratinocytes transiently transfected with a collagenase- or a stromelysin-promoter/CAT reporter gene construct failed to enhance the CAT activity in the presence of IFN-γ. In contrast, mRNA half-life studies utilizing DRB, an inhibitor of transcription, suggested that IFN-γ prolongs the half-life of collagenase mRNA levels, suggesting stabilization of the mRNA. Thus, enhanced stability of the collagenase mRNA could lead to elevated steady-state levels of the mRNA transcript, and subsequent translation of the mRNA to the corresponding protein, as demonstrated by Western immunoblotting analysis, leads to enhanced synthesis of procollagenase. At the same time, stromelysin, which is required for maximal activation of collagenase [3], is coordinately upregulated by IFN-γ in the absence of noticeable changes in the TIMP levels. Thus, these changes are expected to lead to an imbalance between the activity of these MMPs and their principal tissue inhibitor. This situation potentially leads to enhanced degradation of the extracellular matrix of connective tissue in situations involving close cell-matrix interactions, such as wound-healing processes [9].

It is of interest to note that another cytokine, transforming growth factor-β (TGF-β), has been similarly shown to suppress the collagenase activity in fibroblasts, and this inhibition involves two mechanisms [24]. First, the synthesis of collagenase protein is inhibited by TGF-β and, secondly, the synthesis of the inhibitor, TIMP, is upregulated. In contrast, TGF-β elevates collagenase activity in keratinocytes.* These cell type–specific differences suggest differential signal-transduction pathways, because the *cis*-acting elements in the collagenase gene are expected to be identical both in keratinocytes and fibroblasts. In this context, it was of interest to note that the constitutive expression of collagenase has been suggested to involve AP-1, and its corresponding *cis*-acting binding element, TRE [1,2]. In this study, we demonstrated in transient transfections that keratinocytes upregulate the expression of a heterologous promoter, thymidine kinase, when linked to AP-1 responsive element, TRE. However, IFN-γ did not further enhance the activity of this construct $[TRE]_3TK/CAT$. These observations further attest to the possibility that post-transcriptional regulation is responsible for IFN-γ enhanced expression of the collagenase in keratinocytes.

An interesting observation noted in our study was that the upregulation of MMP-1 and MMP-3 gene expression by IFN-γ was shown to be dependent on on-going protein synthesis, because addition of cycloheximide to the cell cultures 1 h prior to the addition of IFN-γ abrogated the IFN-γ effect. This observation contrasts with previous demonstrations of cycloheximide-induced

* Mauviel A, Chen YQ, Dong W, Tamai K, Uitto J: Cell-type specific induction of *c-jun* and *jun-B* proto-oncogenes by TGF-β is responsible for differential regulation of collagenase gene expression in fibroblasts and keratinocytes (abstr). *J Invest Dermatol* 102:528, 1994.

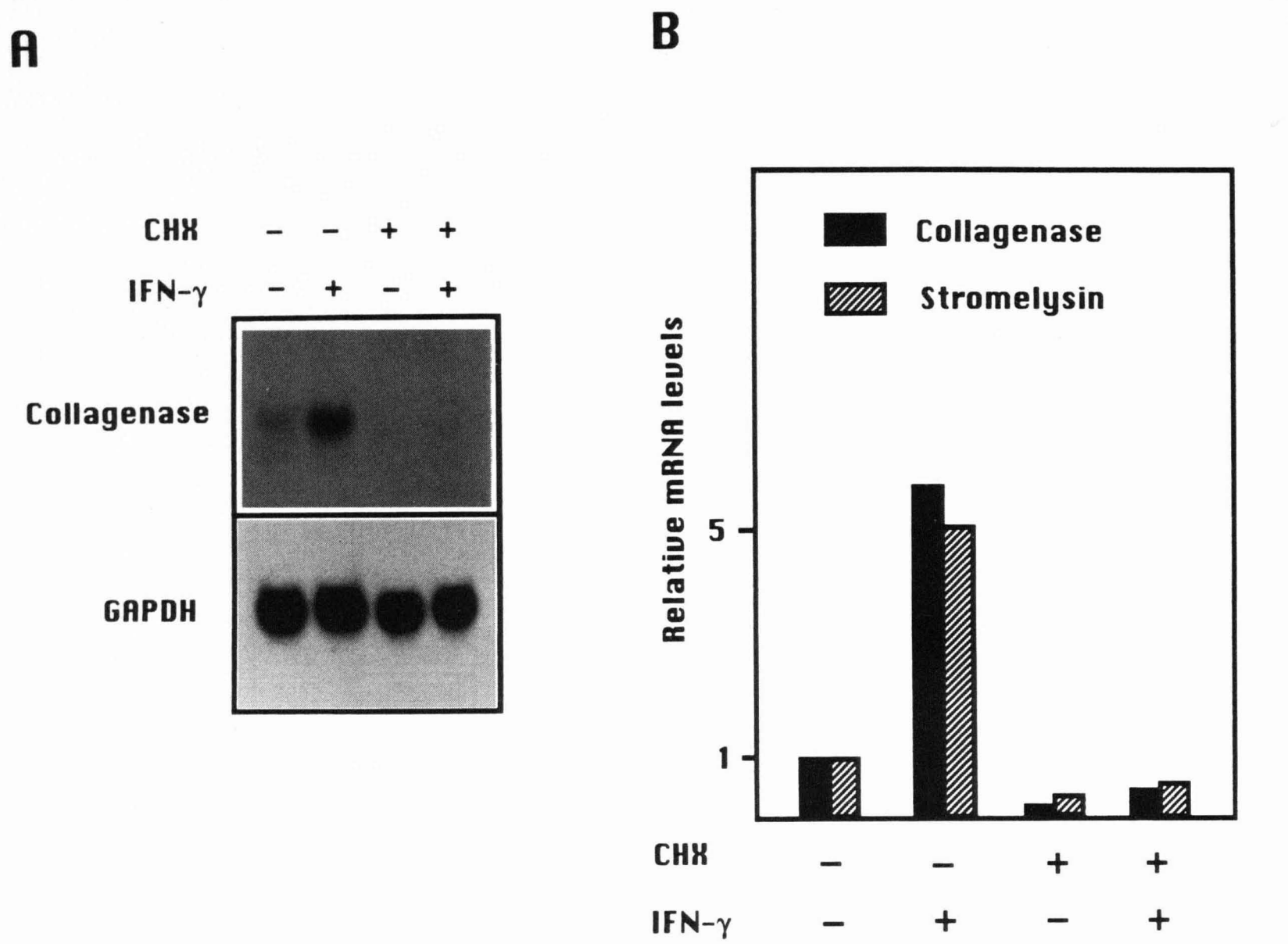


**Figure 3. The effect of interferon-γ on collagenase and stromelysin gene expression at the mRNA level is dependent on on-going protein synthesis.** Cultured keratinocytes were incubated without (−) or with (+) 100 U/ml of IFN-γ in the presence (+) or absence (−) of 10 μg/ml of cycloheximide (CHX), which was added to the cultures 1 h prior to addition of IFN-γ. The steady-state levels of collagenase and stromelysin mRNA were determined, as described in **Fig 1** (*A*), and the relative levels were determined by scanning densitometry after correction for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels (*B*).

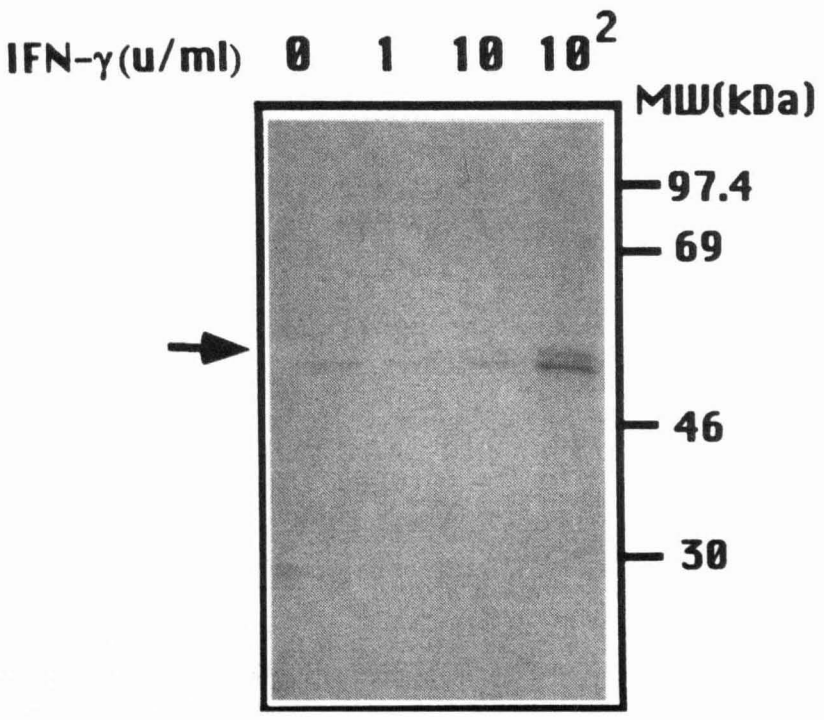


**Figure 4. IFN-γ enhances collagenase (MMP-1) gene expression at the protein level.** Cultured keratinocytes were incubated with IFN-γ in concentrations indicated for 24 h, and the medium proteins were analyzed by Western immunoblotting utilizing a polyclonal anti-human collagenase antibody. Please note the characteristic doublet of bands of ~62 and 57 kDa (*arrow*). The positions of molecular weight markers (MW) are indicated on the right.

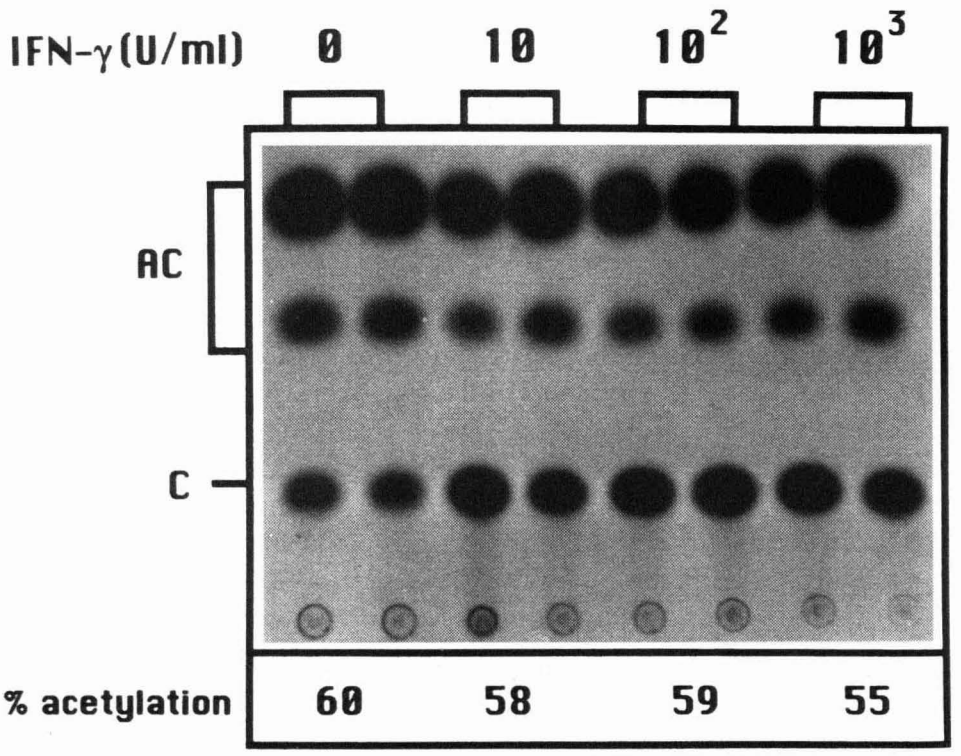


**Figure 5. IFN-γ does not alter the collagenase-promoter/reporter gene construct expression in transient transfections of cultured human keratinocytes.** The cells were transfected with the construct pCLCAT3 containing 3.8 kb of the promoter region of MMP-1 linked to the CAT reporter gene. One hour following the transfection, IFN-γ in concentrations indicated was added, and CAT activity was determined after 24 h of incubation. The acetylated (AC) and non-acetylated (C) forms of [$^{14}$C]chloramphenicol were separated by thin-layer chromatography as described in the text, and CAT activity, expressed as percent acetylation of [$^{14}$C]chloramphenicol, is indicated.

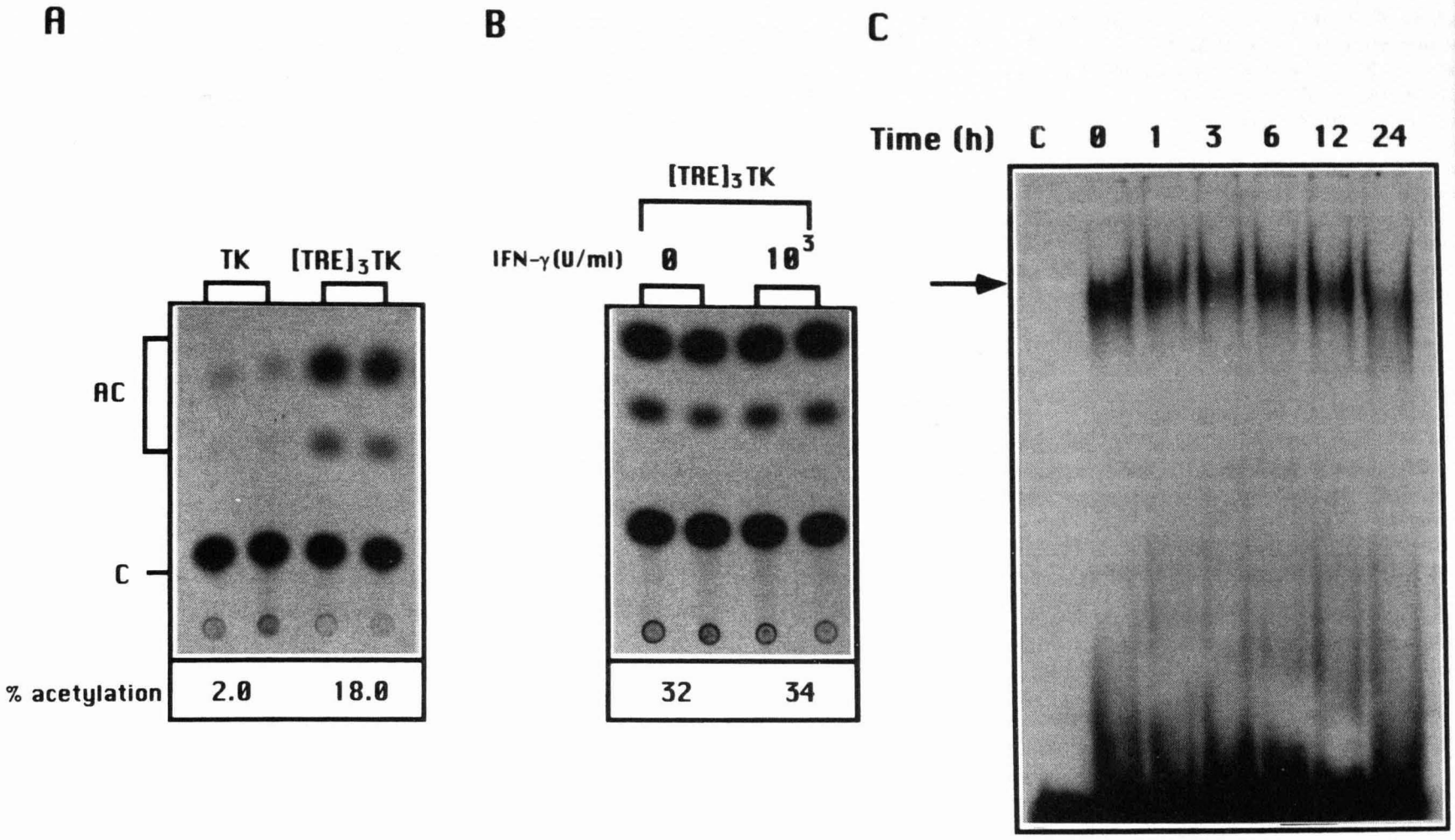


**Figure 6. IFN-γ does not modulate AP-1–driven promoter activity or AP-1–binding activity in cultured human keratinocytes.** *A*: Cultured keratinocytes were transfected in parallel with a thymidine kinase (TK)/CAT construct and a similar construct, $[TRE]_3TK/CAT$, which contained in addition three AP-1–binding sequences (TRE) in tandem in front of the TK promoter. The expression of TK-promoter/CAT construct was markedly enhanced by the presence of three upstream TRE sequences. *B*: Incubation of cells transfected with $[TRE]_3TK/CAT$ construct with IFN-γ (1000 U/ml) did not change the level of expression. Note that *A* and *B* are two separate experiments and, therefore, the percent acetylation in control cultures transfected with $[TRE]_3TK/CAT$ incubated without IFN-γ are different. *C*: Gel mobility shift assay with a radioactively labeled 20-bp oligomer containing the collagenase AP-1 consensus binding sequence (TRE; see *Materials and Methods*), incubated with keratinocyte nuclear proteins, demonstrated a radioactive DNA/protein band (*arrow*). However, this binding activity did not change appreciably during incubation of the cells with IFN-γ (1000 U/ml) up to 24 h. The *C* lane indicates reaction without nuclear proteins added to the binding reaction.

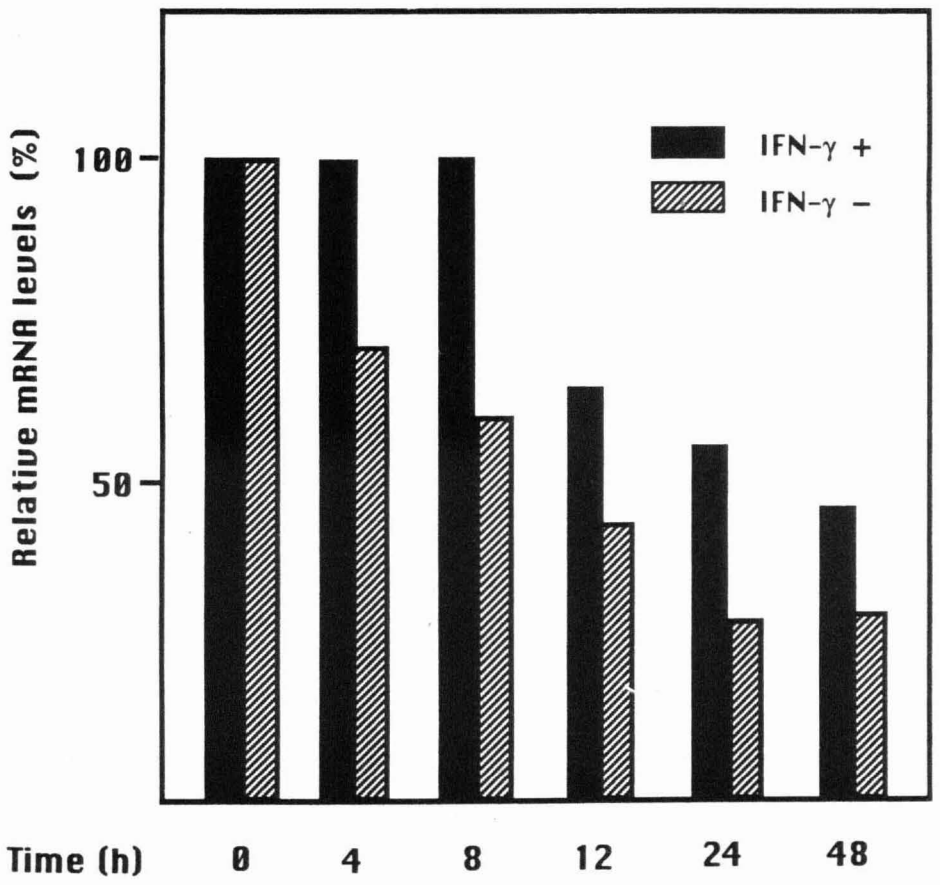


**Figure 7. IFN-γ stabilizes MMP-1 mRNA and prolongs its half-life.** Keratinocyte cultures were incubated without (▨) or with (■) IFN-γ (100 U/ml) for 24 h. Cells were then rinsed with phosphate-buffered saline, and fresh medium containing 60 mM DRB was added (time 0 h). Total RNA was then isolated from parallel cultures at time points indicated, and the MMP-1 mRNA levels were determined by Northern hybridizations. The values are expressed as percent of the mRNA levels in 0 hour cultures, after correction for glyceraldehyde-3-phosphate dehydrogenase mRNA levels.

stromelysin mRNA levels in cultured human fibroblasts [22]. These observations further emphasize the differences noted in different cell types with respect to IFN-γ effects on the regulation of these MMPs. Collectively, these results provide intriguing novel evidence for the role of the cytokine network in regulating the extracellular matrix turnover in physiologic and pathologic situations.

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